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Histological Study of Hair Follicles Treated With a 3-msec Pulsed Ruby Laser

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Background and Objective: **Ruby laser energy at 694 mn is moderately absorbed by melanin and minimally absorbed by other skin chromophores. This property and its depth of penetration into dermis permit absorption into pigmented hair follicles, thus making it suited to photothermolysis of these appendages. Clinical reports of the efficacy of such lasers for removal of unwanted hair are emerging in large numbers, but scientific data regarding the exact mechanism of action is still lacking. This study aims to evaluate and define further the histological responses of hair follicles to 3-msec pulsed ruby laser light.**
Study Design/Materials and Methods: **Twenty-four patients with brown or black axillary or groin hair were treated with a 3-msec ruby laser at fluences from 10 to 40 J/cm^2 on one, two, or three occasions. Biopsies were taken at various intervals from immediately to 8 weeks after treatments. Biopsies were fixed and stained with either nitroblue tetrazolium chloride or hematoxylin and eosin for histological examination.**
Results: **One treatment induced changes typical of catagen followed by telogen at all fluences. The papillae always remained viable. Two and three treatments resulted in atypical telogen, with infundibular dilatation and plugging, and marked proliferation of the stem outer sheath. New anagen follicles were evident even after three treatments at 12- and then 8-week intervals and were biopsied 6 weeks later, but there were no hairs extending to or through the epidermis.**
Conclusion: **There was no evidence of permanent follicle death after one ruby laser treatment. However, despite evidence of persistence of follicular elements after two and three treatments, it is possible that laser-induced damage to the isthmus and upper stem may interfere with the interaction between dermal and epidermal germinative cells, thus inhibiting or altering the normal hair cycle. Lasers Surg. Med. 24:142–150, 1999.**

Key words: epidermis; dermis; hair removal; melanin; papilla

INTRODUCTION

Since the incidental finding of a decrease in the density of terminal hair during ruby laser tattoo removal, there has been an explosion of lasers released onto the world market purporting to destroy hair selectively. The proposed mechanism of action is absorption of laser light by a chromophore within the follicle. In the case of ruby and alexandrite lasers and high intensity pulsed light sources, the melanin in the shaft of the follicle is claimed to be the target chromophore. When Nd:YAG lasers are used, the chromophore is sup-

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posedly created by first epilating the hair from the follicle and then filling the empty follicle with a carbon-containing substance.

Despite the rapidly expanding variety of lasers flooding the hair removal market, there have been few published studies illustrating the mechanism of action of these lasers, especially histological responses [1]. Most studies to date have concentrated on evaluation of efficacy of treatment by hair counts [2–5], a method that is inherently flawed if the period of posttreatment observation is less than the known period of natural telogen for that region of the body. In addition, animal studies may only be approximated to the human situation in a guarded and limited fashion. Differences in the behavior of hair follicles between species have been well documented [6].

This study employs a laser claimed to be purpose-built to approximate the theoretical parameters necessary to destroy terminal hair selectively while sparing the epidermis from thermal damage [5,7]. We evaluate the histological responses of axillary and groin skin to the full range of energies of this laser at intervals from immediately after to 8 weeks after one, two, and three treatments.

MATERIALS AND METHODS

The Laser

The laser was a Spectrum Epilaser™ long-pulsed ruby laser emitting light at 694 nm in pulses of 3 msec at pulse intervals of 2 sec. The spot sized used was 7 mm or 10 mm, with light delivery via an articulated arm and lensed handpiece. The method of treatment was the standard method recommended by the manufacturer, which is using the contact mode with the sapphire endpiece pressed against the skin for approximately 0.5 sec prior to laser irradiation. The handpiece was then removed from the skin and placed adjacent to the previous site, with an overlap of about 30%. The sapphire end was cooled to 4°C for each laser impulse.

Patients

Twenty-four informed-consenting volunteers entered the study, 15 in phase 1, 9 in phase 2. Only those with brown or black hair and Fitzpatrick skin types I–III were included. The areas treated were either the axilla or the groin according to patient preference, with 7 patients choosing the groin and 17 the axilla.

Methods

In both phases of the trial, patients avoided any form of epilation except shaving 1 month prior to commencement of the trial and demonstrated at least 3 days of hair growth from the last shaving to initial and each subsequent assessment.

The area to be test treated was outlined and photographed, with the fluences to be used marked on the skin with red ink. The treatment area was then shaved immediately prior to treatment.

Phase 1 (15 patients). Each patient was treated with five different fluences: 10 J/cm^2, 15 J/cm^2, 20 J/cm^2, 30 J/cm^2, and 40 J/cm^2. Each patient underwent one biopsy, either immediately after treatment, 1 week later, or four weeks later. Because there were five patients per biopsy group, a biopsy was taken of skin treated at each of the five energies and at each of three intervals after treatment. Biopsies taken immediately after treatment were snap frozen and prepared for nitroblue tetrazolium chloride (NBTC) enzyme stain to assess cell viability. All other biopsies were routinely fixed in formalin for hematoxylin and eosin (H&E) staining. Biopsies were examined by a pathologist who was aware that the skin had been treated with a ruby laser but was blinded to the fluence used.

Of these 15 patients, five had an immediate biopsy and NBTC stain, five had biopsy at 1 week and H&E stain, and five had biopsy at 4 weeks and H&E stain.

At the time of biopsy, the treated area was photographed and the patients were instructed to refrain from shaving for 3 days prior to the follow-up visit.

Phase 2 (9 patients). Each patient was treated with three different fluences: 20 J/cm^2, 30 J/cm^2, and 40 J/cm^2. Three patients were biopsied 8 weeks after one treatment only, three patients were biopsied 6 weeks after two treatments 12 weeks apart, and three patients had two treatments 12 weeks apart and one treatment 8 weeks after the second treatment and were biopsied 6 weeks after the third treatment.

RESULTS

Phase 1

Immediate biopsies, NBTC and H&E stains. All of the biopsies showed damage to the intraluminal hair shaft, with clefting, homogeni-

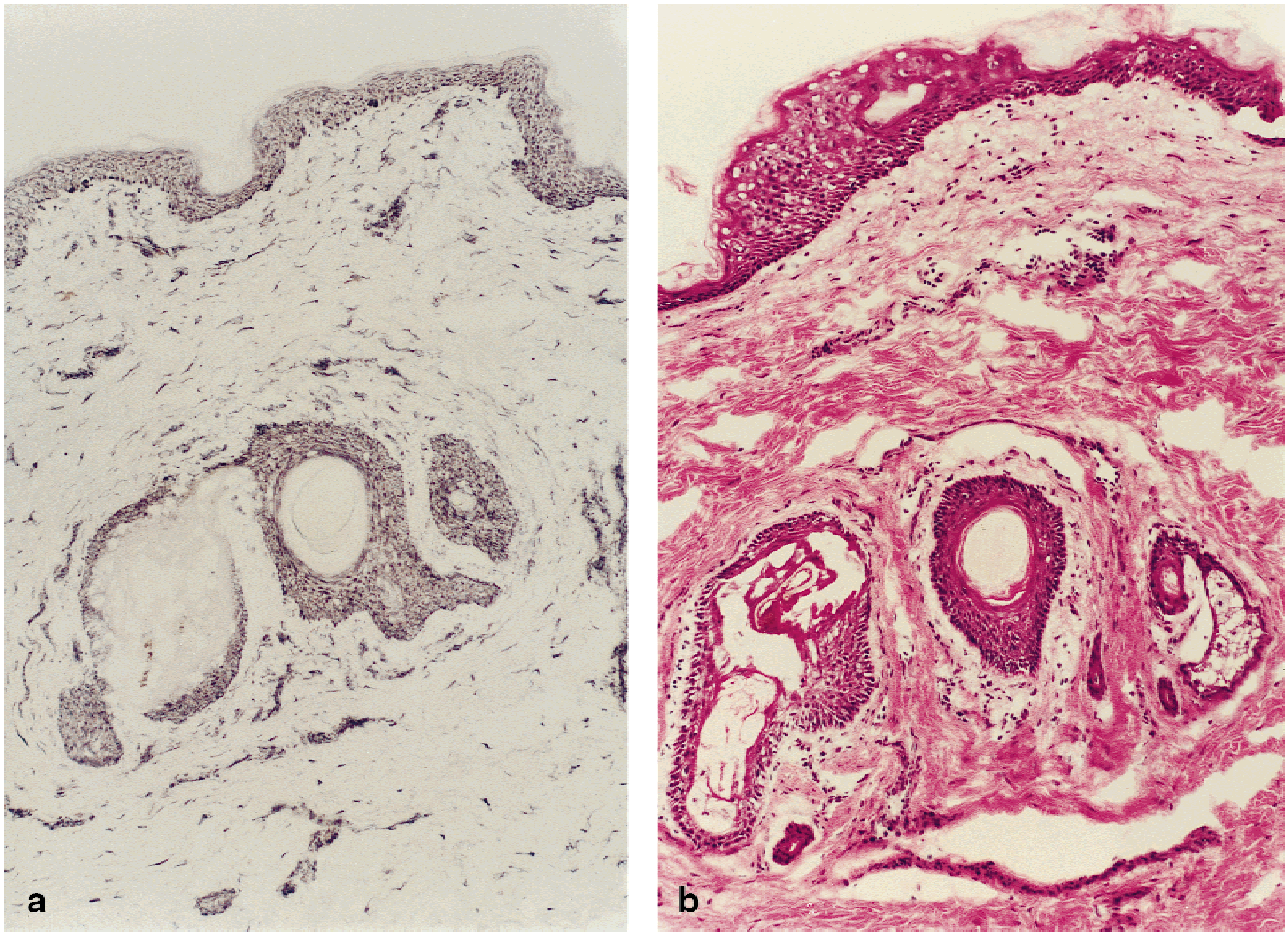

Fig. 1. **a,b:** Nitroblue tetrazolium chloride and hematoxylin and eosin stains of three follicles sectioned obliquely through the isthmus of follicles at the level of the sebaceous glands treated at 30 J/cm^2. There is pallor of the inner sheath cell layers in the terminal hair follicle on the left, but the telogen and vellus hairs nearby are not affected.

zation, and some streaking of melanin pigment on H&E stain. The severity of these events increased with increasing fluences. On enzyme staining, all specimens showed a similar reaction, with only the severity of the changes increasing as the fluence increased. The inner root sheaths were pale, suggesting loss of cell viability in this zone, but in all cases except at 40 J/cm^2 the outer sheath remained viable, and the lower follicular bulbs including the papillae demonstrated viable cells at all fluences. The epidermis and dermis otherwise appeared normal (Figs. 1, 2).

Biopsies at 1 week, H&E stains. The consistent finding at all fluences was eosinophilic homogenization of the intraluminal hair shaft, with melanin pigment smearing and variable cuticle disruption (trichomalacia). The general appearance of the laser-irradiated hair follicles was one of early catagen, with slight thickening of the vitreous basement membrane, apoptotic cells in the outer root sheath, and degeneration of melanocytes with pigment incontinence. Only the severity of these changes increased with increasing fluence. At this stage, there were no fibrous tracts typical of "normal" catagen. Where telogen or vellus hairs were included in a biopsy, these follicles appeared unaffected by laser irradiation. The epidermis appeared normal, but the dermis showed a low-grade, mixed inflammatory infiltrate of lymphocytes, histiocytes, and eosinophils surrounding the vessels of the superficial and deep dermis.

Biopsies at 4 weeks, H&E stains. In all biopsies at all fluences there was a consistent finding of hair follicles appearing in late catagen/telogen phases (Fig. 3). The luminal hairs showed focal homogenization, trichomalacia, and pigment casts, and numerous apoptotic epithelial cells were within the sheath. Fibrous streamers perfo-

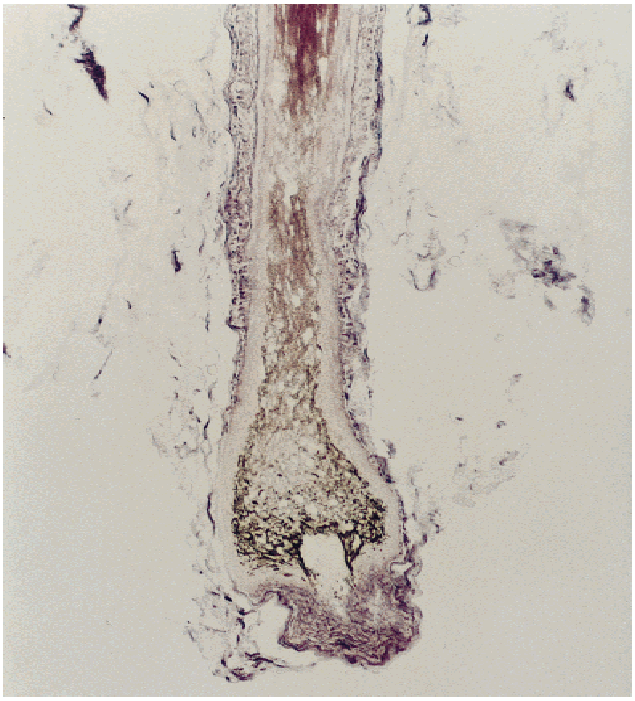

Fig. 2. Nitroblue tetrazolium chloride stain of a terminal anagen hair treated at 20 J/cm^2. There is marked pallor signifying nonviable cells in the inner root sheath. The matrix of the bulb shows black-staining melanin but no blue staining of living cells. However, the base of the bulb and papilla and the outer root sheath calls all are viable.

Fig. 3. A follicle treated at 40 J/cm^2 and biopsied 4 weeks later. The lumenal hair shaft shows trichomalacia and pigment casts. The follicle is in telogen, but a follicular germ and incipient papilla herald the commencement of very early anagen.

rated by small blood vessels and melanophages were seen extending into the deeper dermis beneath the elongated lower epithelial segments of the involuting hairs. At 40 J/cm^2, the hairs were almost at telogen, with a dermal papilla contacting but not indenting the ascending hair bulbs. The epidermis and dermis appeared normal.

Phase 2

One treatment, biopsies at 8 weeks, H&E stains. At all three energies, both terminal and vellus hairs could be seen in all biopsies. The vellus hairs appeared essentially normal except one (at 40J/cm^2) in which the inner root sheath of the stem was mildly homogenized with a reduction in corneocyte numbers. Terminal hairs were either in anagen or late catagen/early telogen, with perhaps a greater proportion of catagen/telogen to anagen hairs than would normally be expected in untreated skin (four anagen and two catagen in the 40J/cm^2 biopsy, two anagen and two late catagen/early telogen in the 30 J/cm^2 biopsy, but only anagen hairs in the 20J/cm^2 biopsy). All three biopsies showed a mild, perivascular, mixed chronic inflammatory infiltrate of histiocytes, lymphocytes, and mast cells throughout the dermis. The epidermis appeared normal.

Two treatments 12 weeks apart, biopsy at 6 weeks, H&E stains. Biopsies at all three energies showed similar findings. There was cystic dilatation and plugging of the infundibulum of all terminal hairs. Deeper in the dermis the follicles were either in telogen or early anagen, although there were no hair shafts protruding from surface follicular openings (Fig. 4a,b). However, there were no dense fibrotic scar tracts indicative of dropped-out hair follicles. At the lowest fluence (20 J/cm^2), a retracting catagen hair showed early budding of the outer root sheath. The superficial dermal vessels in all specimens showed a low-

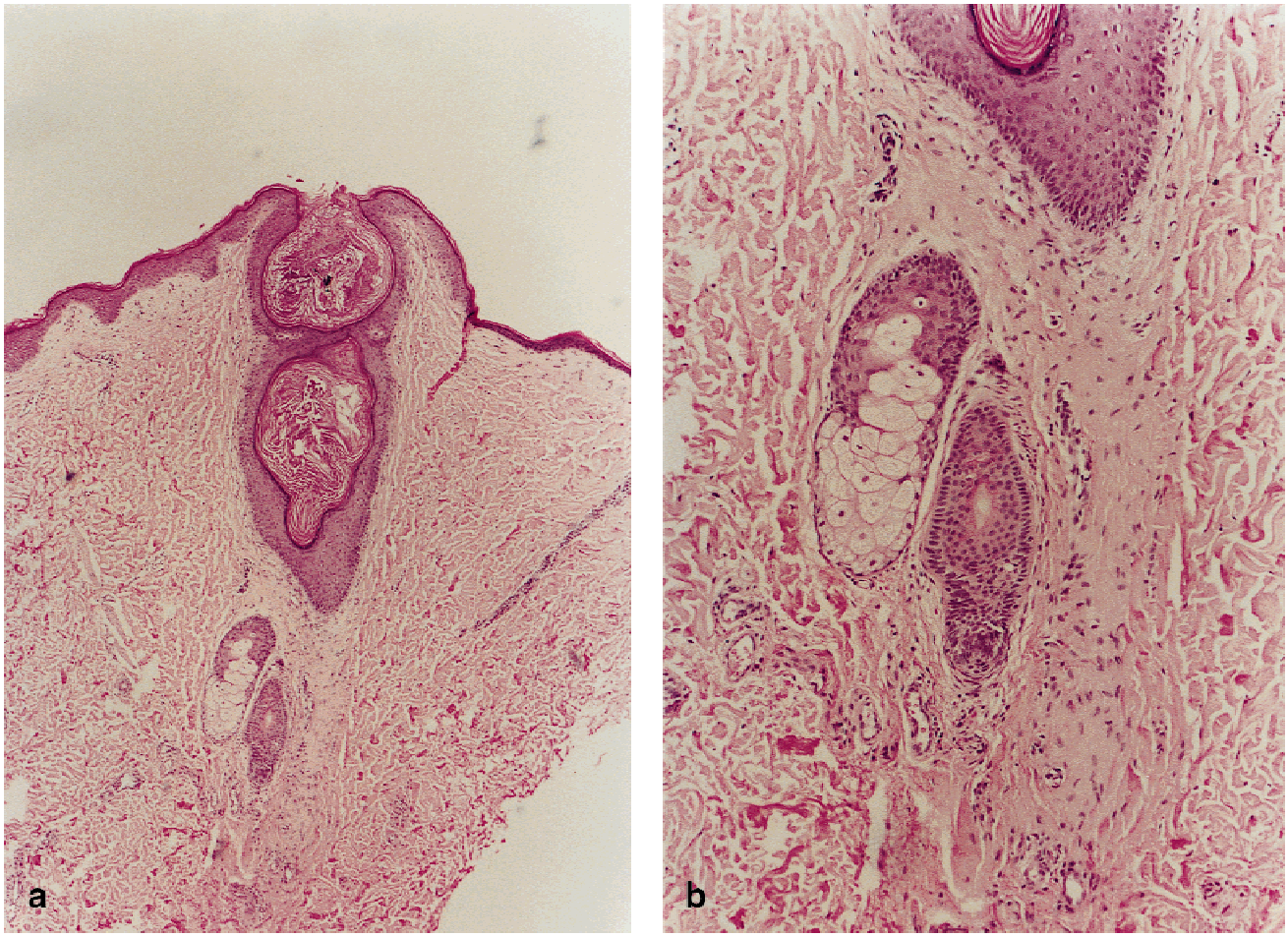

Fig. 4. **a,b:** After two treatments at 40 J/cm^2 and then a biopsy 6 weeks later. The superficial infundibular region of the follicle is dilated and plugged with keratin. Melanin remnants are visible. The lower portion of the follicle shows the newly forming matrix of a very early anagen hair.

grade, lymphohistiocyic, perivascular imflammatory reaction.

Three treatments, the 2nd at 12 weeks, the 3rd 8 weeks later, biopsies 6 weeks afterward— H&E stains. All terminal hairs were in late catagen/early telogen or in early anagen. There were no mature anagen follicles. Where infundibula were sectioned, they were seen to contain nonpigmented miniaturized hair shafts. However, there were no dense fibrotic tracts suggestive of scarred hair follicles, and early anagen hairs deep in the dermis were clearly developing regardless of the fluence used in treatment. Telogen hairs showed striking budding of the outer root sheaths (Fig. 5), and in one biopsy (40 J/cm^2) a single multinucleate giant cell was visible deep to a hair papilla and matrix (Fig. 6). Dermal vessels appeared minimally telangiectatic, and a mild perivascular lymphocytic reaction was seen in addition to a mixed lymphocytic/histiocytic/plasma cell infiltrate in the deep dermis localized around apocrine glands in one patient.

Assessment of Clinical Photographs

Although pre- and posttreatment photographs were taken throughout both phases of the trial, no hair counts or blinded analyses were performed. Anecdotally, there was almost complete regrowth of hair in all patients in phase 1 after 8 weeks, although there was some suggestion that the hair was finer; in phase 2 of the study 6 weeks after two and three treatments, there was a marked reduction in hair regrowth. This reduction was a laser-induced event because regrowth was evident in narrow strips between zones treated at different fluences (Fig. 7a–d). Five patients developed hyperpigmentation in zones

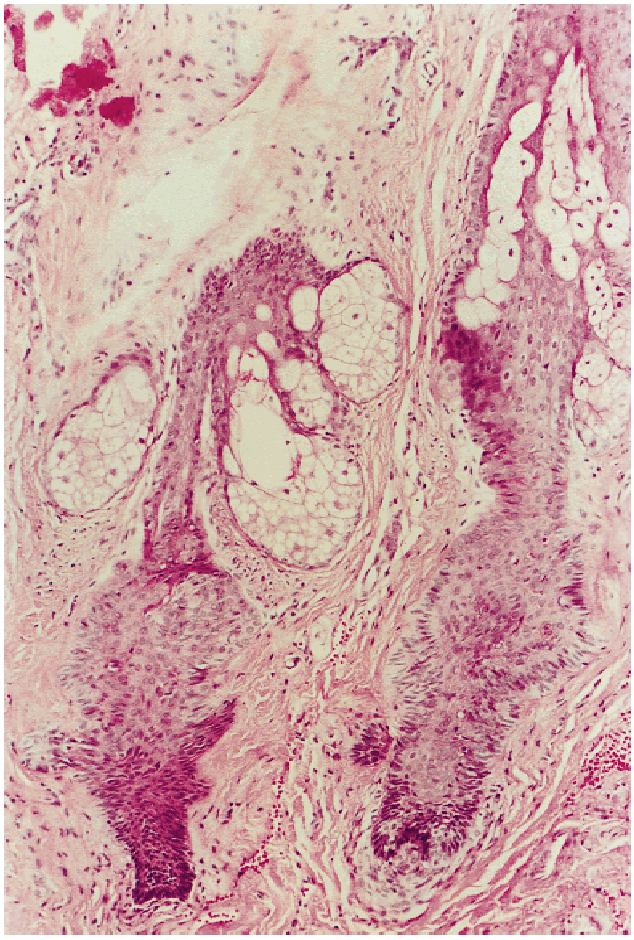

Fig. 5. Three treatments at 30 J/cm^2, 12 weeks and then 8 weeks apart, and biopsy 6 weeks later. Both folllicles show budding of the outer root sheaths and, at their bases, newly forming follicular germs, on the right, indented by a dermal papilla, indicative of early anagen.

treated at 40 J/cm^2. This was secondary to a moderate inflammatory reaction and some mild crusting in the weeks following treatment. All of these instances were in patients with type III skin. One patient developed some patchy hypopigmentation in the regions treated at the highest fluence. Despite these clinical observations, there was no histological evidence of significant epidermal damage, although a low-grade dermal lymphohistiocytic reaction was observed in most biopsies.

DISCUSSION

At all energies in phase 1 there was evidence of damage to the hair shaft and inner root sheaths that increased in severity with higher fluences. This was clearly seen on the NBTC-stained biopsies sampled immediately after treatment. However, even at the highest fluences, there was no evidence of total follicular death. The papillae remained viable in all instances. The predominance of catagen hairs at 1 week and late catagen/early telogen hairs at 4 weeks implies that laser treatment induces this process.

Phase 2 biopsies at 8 weeks after only one treatment showed some anagen hairs that had no obvious signs of laser-induced changes but a greater proportion of late catagen/early telogen hairs than would be expected in untreated skin (normally 2% of hairs are in catagen, 10–15% in telogen). No biopsies showed any signs of dense fibrotic tracts of scarred hair follicles that might be expected if laser irradiation of follicles had caused permanent follicular death after one treatment. Two conclusions may be drawn from these observations. First, because the laser appears to induce follicle cycling, we assume that any hairs that have been irradiated should be in late catagen or telogen 4–8 weeks after one treatment. Any anagen hairs present may represent follicles that escaped adequate irradiation at the time of treatment due to gaps in spot overlap or they may be hairs that were in telogen at the time of treatment and therefore escaped light energy absorption due to absence of contained chromophore. Second, clinical studies in which evaluations of hair growth reduction have been made at posttreatment intervals of less than 8 weeks, and probably considerably longer, may be considered inconclusive. Hair shafts protruding through the skin, or the lack thereof, at this posttreatment interval can give no indication of the efficacy of this treatment as a long-term or a permanent hair removal method.

Follicles treated twice with an interval of 12 weeks were similar to those treated three times with intervals of 12 and then 8 weeks when biopsied 6 weeks later. Both showed cystic dilatation of the infundibulum with keratin plugging and an absence of hair shafts in these follicles but no suppurative or granulomatous folliculitis. Both showed a range of phases of follicles, although there was a predominance of late catagen, telogen, or early anagen hairs. Mature normal anagen hair follicles were sparse. The significance of the single giant cell seen in one biopsy after three treatments is uncertain. Previous unpublished histological studies performed after treatment with this laser have suggested that granulomatous degeneration is one of the mechanisms of

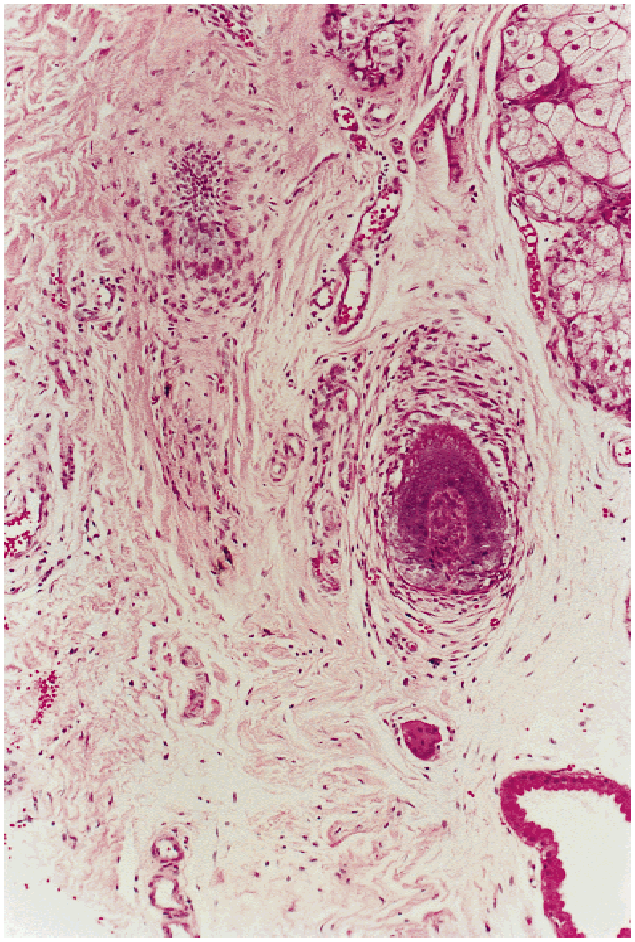

Fig. 6. One multinucleate giant cell and a few pigmented macrophages sit below a dermal bulb and papilla, signifying prior follicular damage in this area. This biopsy was taken from the axilla of a patient treated at 40 J/cm^2 three times: two treatments 12 weeks apart and 8 weeks after the second treatment.

hair follicle drop-out (Prof. R. Rox Anderson, Wellman Laboratories, Boston, July 1997, personal communication). However, our biopsies did not confirm this possibility.

In the biopsies taken of hairs treated three times, there was persistent evidence of early anagen hairs even in biopsies of skin treated at the highest fluences. This finding supports the assumption made from biopsies taken immediately after treatment that the papilla and base of the bulb are not sufficiently seriously injured to prevent a new follicle from developing. Hairs treated two and three times, especially those treated at 30 and 40 J/cm^2, showed striking budding of the outer root sheath, a phenomenon that is sometimes seen in sheep when seasonal changes and nutritional deficiencies cause an increase in the percentage of wool follicles that shed fiber and "collapse," thereby leaving an empty follicle devoid of inner sheath components and an outer sheath that closes in to occupy the space once taken by the fiber and inner sheath layers [8]. Plugged dilatation of infundibula is also seen in this circumstance. When these follicle changes are seen in sheep, regrowth of those hairs follows when living conditions for the animal improve. These changes were only seen in any follicles treated more than once in our study. The presence of early anagen bulbs in all biopsies, even those treated three times and at high fluences, should imply that hair regrowth will occur, but our clinical photographs confirm progressively more convincing reduction or delay in hair regrowth with each treatment (Fig. 6a–d). In addition, our clinical impressions over 18 months of experience with this laser confirm that there is a very obvious reduction in hair regrowth after a minimum of two treatments that is sustained for more than 1 year. It should be emphasized that after only one treatment we found little if any detectable reduction in hair density, an observation that correlates with our phase 1 histological findings of apparently reversible follicular injury.

Clinical studies showing anything from 41% reduction in facial hair 12 months after one ruby laser treatment to 71% reduction on legs 3 months after an unspecified number of treatments with a ruby laser were included in no less than 14 oral and six poster presentations at the 1998 American Society for Laser Medicine and Surgery Annual Meeting. A plethora of clinical results using a variety of lasers and pulsed light sources on different anatomical regions was presented. However, none showed clinical results longer than 12 months after the last treatment, and most follow-up periods were between 3 and 6 months. Given that naturally occurring telogen is 3–73 weeks (approximately 1–18 months) on different parts of the body [9] and that laser treatment may induce synchronized telogen of laser-irradiated follicles rather than follicular death, clinical follow-up periods must necessarily be extended past 18 months especially in areas such as arms (telogen 8–24 weeks), legs (telogen 8–38 weeks), and the pubic region (telogen 51–73 weeks) before meaningful conclusions can be made regarding the permanence of hair removal.

Despite the present lack of convincing long-term clinical evidence of permanent hair loss after laser treatment, we postulate that permanent hair removal may be possible after two or more

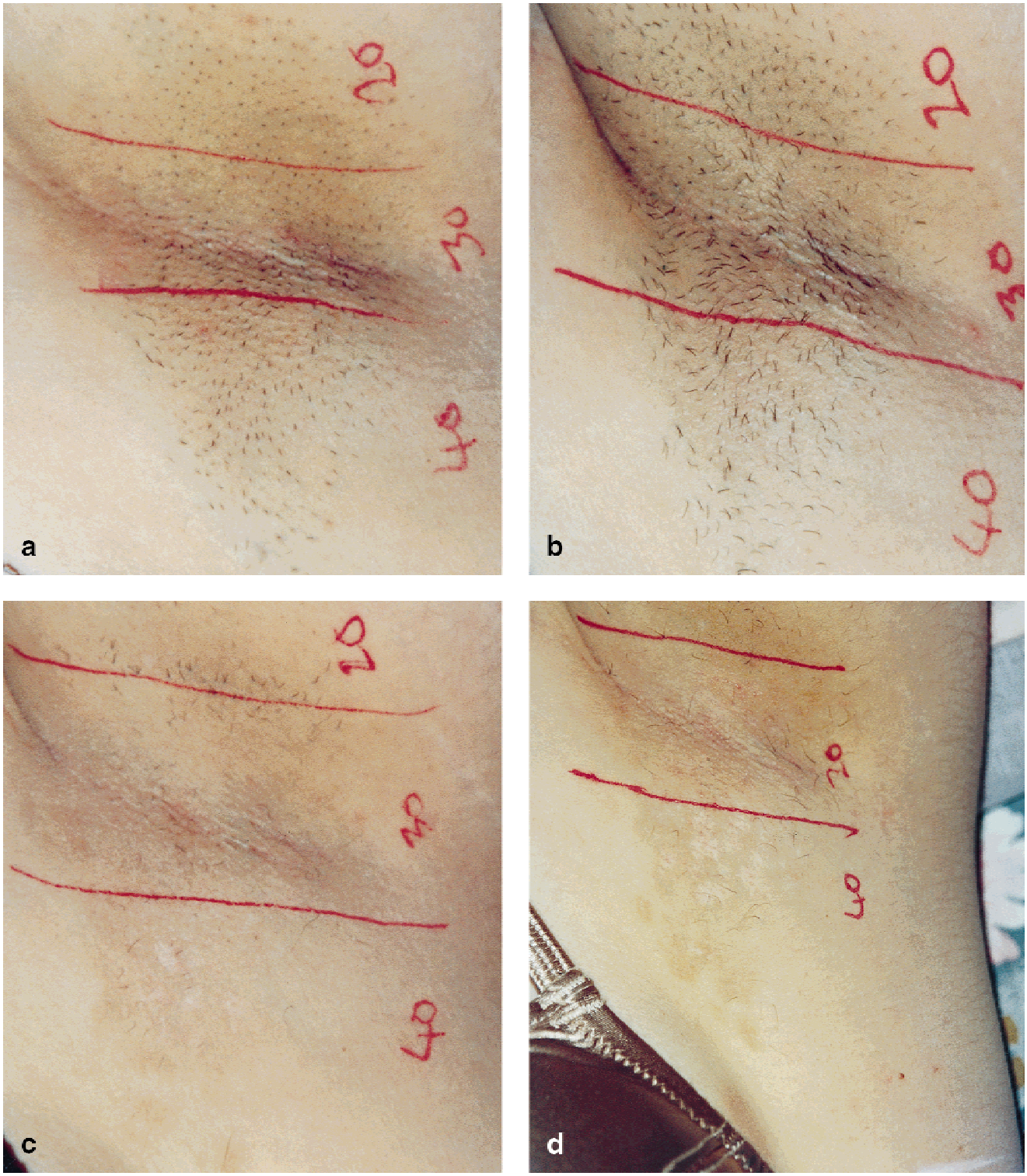

Fig. 7. The axilla of the same patient from which the biopsy shown in Figure 5 was taken. **(a)** Before treatment. **(b)** Twelve weeks after one treatment. **(c)** Eight weeks after two treatments. **(d)** Six weeks after three treatments, immediately before biopsy of the area treated at 30 J/cm^2.

treatments despite the appearance of new anagen hair bulbs in the dermis. Retreatment at 8–12 weeks must impact on follicles in late catagen/telogen phases induced by the first treatment. Presumably it is this event that results in the changes seen after two and three treatments, which are quite different from those seen after only one treatment. It is possible that repeated laser light absorption into the residual damaged hair shaft in the isthmus and upper stem induces enough damage to this portion of the follicle to separate the papilla from the infundibular epithelium. It would seem that retreatment of these follicles in late catagen/telogen causes a "sick follicle" appearance typified by infundibular dilatation, discontinuity between the infundibular epithelium and the papilla, and exaggerated budding of the outer root sheath of the lower follicular stem.

Animal research has demonstrated that isolated papilla cells grown in vitro are capable of developing into new hairs but only if transplanted into skin in contact with epithelial cells [10]. Also, transected sheep follicles grown in culture will attempt to form a new bulb from the residual remaining upper two-thirds of the shaft, although they do not grow a hair [6]. When human hair shafts transected to remove the bulb have been transplanted into skin, they reform a dermal papilla and produce hairs, although the fiber is finer than the parent hair [11]. The interactions between dermal and epidermal germinative cells of hair follicles are complex and as yet incompletely understood. The minimum number and type of germinative follicular cells necessary to produce a mature terminal hair in human skin are not exactly known, although stem cells in the outer root sheath appear to play an important part. We postulate that it is the interference with the normal dermal–epidermal stem cell interactions that is the mechanism by which the laser acts to interrupt the hair follicle cycle.

At this time, no conclusions can be drawn with regard to subsequent events regarding the fate of the newly developing hair bulbs we have seen deep in the dermis. Biopsies immediately after a second treatment and additional biopsies at 4, 5, and 6 months after one and two treatments may futher elucidate the progress of histological events after treatment of hair follicles with the Epilaser™.

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